Various aspects of the microbiological quality of pharmaceutical preparations.

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In chapter 1 the factors are discussed that determine the proliferation of micro-organisms in pharmaceutical preparations and the subsequent contamination and infection of the user/patient. This discussion concerns (i) the possibly pathogenic and toxin forming micro-organisms, (ii) the micro-organisms present in used and non-used pharmaceutical preparation and (iii) the microbial deterioration of pharmaceutical preparations.

The intrinsic factors e.g. a_w, pH and presence of nutrients determine the ecology as well as the extrinsic factors, such as temperature and design of package. It is very difficult to predict the effect of a preservative, because of the complex formulation of most pharmaceutical preparations. Therefore, the effect has to be tested.

Although some aspects of the manufacturing process are evaluated within this thesis, GMP is not discussed. The non-obligatory requirements of the Ph.Ned. VIII concerning the microbiological quality are mentioned at last and the objectives, the scope and the historical background of this thesis are described.

In chapter 2 the statistical reliability of the poured plate count is evaluated. The optimum estimator is derived and for the estimation of the variance the counting errors, dilution errors, sampling errors and mixing imperfections are taken into account. This estimation appears not to be completely reliable, because some error sources must have been overlooked.

The technique of the MPN and a single series assay are described.

The standard (two way) analysis of variance is adapted, because the recoveries that must be tested are not independent within one cell and consequently the different recoveries are calculated using the same inoculum. Physico-chemical factors determining the recoveries are calculated. Therefore, the recovery is calculated

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Some microbiological techniques are described as well, such as the taxonomy of Gram negative rods and Gram positive bacteria. These techniques are taken from the various pharmacopoeias as far as possible. This concerns *Pseudomonas aeruginosa*, *Staphylococcos aureus*, *Escherichia coli* and *Salmonella* species. All isolated Gram negative rods are identified by means of the API 20E system and the Gram positive bacteria are classified with methods, that are usually applicated in the Microbiology of Food. These classified species are identified as far as it concerns *Staphylococcus aureus*, *Bacillus cereus*, Lancefield group D streptococci and some coryneform bacteria. These techniques are applicated in the following chapters.

In chapter 3 studies are made on the suitability of some procedures used to test the microbiological quality of dermatological preparations. Isopropyl myristate (IPM) applicated in the membrane filtration method is not suitable, because (i) in some cases the filters are obstructed and (ii) IPM is toxic for *Pseudomonas aeruginosa* ATCC 15442. Purification of IPM does not result in a lower toxicity. The MPN technique appears to be less toxic for *Pseudomonas aeruginosa* than a poured plate technique. Some curves show "tailing".

The method using direct inoculation is tested on some critical points. The method described in Ph.Ned. VIII appears to yield recoveries that differ for different preparations and different microorganisms. It is proposed to relate methods and preparations more directly than has been done up to now in Ph.Ned. VIII.

It is possible to attain stable test emulsions using brain heart infusion (BHI) with 3% $^{w}/_{v}$ lecithin. This fluid is not injurous and after a delay of 3 hours the numbers of cfu are not increased to that amount that the proposed limits of the Ph.Eur. will be exceeded.

In chapter 4 the microbiological quality of pharmaceutical raw materials is investigated. The techniques described in the Ph.Ned. VIII are basically used and the results are compared with the requirements of the annex of the Ph.Ned. VIII and with the proposed requirements of the Ph. Eur. The isolated Gram negative rods are identified by means of API 20E and the Gram positive bacteria are taxonomically grouped.

The raw materials are delivered by 3 wholesalers, but the differences are not significant.

Pseudomonas aeruginosa, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* species are not present.

With respect to the mentioned requirements 14 of the 33 investigated specimens do not pass the tests. The quality of aluminii et magnesii silicas, pancreatinum, thyreoidum, amylum solani, amylum tritici and methylcellulosum is sufficient.

Two-third of the isolated strains of Gram negative rods are identified. *Enterobacter agglomerans*, *Enterobacter cloacae*, *Enterobacter sakazakii* and *Klebsiella pneumoniae* occur most frequently. Four *Pseudomonas* species are isolated. Lancefield group D streptococci or *Bacillus cereus* do not occur in only 3 materials, viz. aluminii et magnesii silicas, talcum and amylum tritici. It is questionable that the various pharmacopoeias do not contain tests to limit the presence of these Gram positive bacteria.

It is concluded that the use of almost all investigated materials is only safe if preservation and the manufacturing of the final products are good. Ignorance on this subject may lead to heavily contaminated final products.

In chapter 5 the microbiological quality of antacid liquids is investigated. The formulation, presently described in the FNA, suffices. The availability of methylparaben (MOB) is approx. 90% and its hydrolysis at $4^{o}C$ is approx. 10% within 100 days. At pH values

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lower than 8.37 Gram negative rods are not present in the antacid liquids. Therefore, it is suggested to dispense these liquids with a pH < 8.37. The dissociation of MOB (pKa = 8.37) and the consequently lower effect are responsible for this phenomenon. Moreover it appears that Gram negative rods may decrease the concentration of MOB and that the concentration of MOB influences the number of bacteria.

After manufacturing according to the old process Gram negative rods such as *Citrobacter freundii*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Pseudomonas fluorescens* occur as well as the Gram positive rods *Bacillus cereus*. Manufacturing according to the new method yields no Gram negative rods, but *Bacillus cereus* and Lancefield group D streptococci occur. It is questionable that these micro-organisms are not limited in the requirements of Ph.Ned. VIII.

In chapter 6 the quality of the preservation of eye drops FNA is investigated. The antimicrobial effect of the basic solutions is tested. The solutions containing benzalkonium chloride (BAC) fulfil the requirement of being sterile within 1 hour after inoculation with 10^6 cfu of *Pseudomonas aeruginosa*. However, the solutions containing phenylmercuric borate (PMB:0.002% and 0.004%) do not meet this requirement at all. Results are gathered using the mean microbicidal effects and using "killing-times".

The influence of added hypromellose is too small to be measured in a killing process.

The addition of pharmacologically active substances to the basic solutions has no significant effect.

Pseudomonas aeruginosa is killed within 15 min. in BAC-containing solutions and within 1 day in the PMB-containing solutions. Other test organisms (2 strains of *Staphylococcus aureus* and *Klebsiella pneumoniae*) are killed within 2 hours in case of BAC-containing solutions and within 1 week in the PMB-containing solution. A spore containing inoculum of *Bacillus subtilis* is not killed

within 1 week (PMB and BAC).

It has no sense to add phenylethanol(PEA) to BAC-containing solutions because they already possess good preserving properties. The addition of a combination of PEA and EDTA to PMB-containing solutions results in a significant increase of the preserving properties. It is suggested that PMB and EDTA are the antimicrobial substances that are potentiated by PEA.

Although PEA takes internationally the third place of the most used preservatives, in the Netherlands some irritations are reported. Hence, investigations on this issue must be completed.